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Please cancel claims 16 and 17.

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#### REMARKS

In response to the Office Action mailed June 28, 2002, please consider the above amendments and the following remarks. Claims 1-13 and 15-17 are pending in the present application, claims 16-17 having been withdrawn as being directed to a non-elected invention. Claims 1-13 are pending and examined on their merits. Claims 7 and 9 are amended and claims 16 and 17 canceled herewith. Applicants acknowledge the withdrawal of certain of the previous rejections and respectfully request entry of the amendments, reconsideration and withdrawal of the remaining rejections.

#### The Invention

The invention provides an improved method for preparing a nucleic acid array, in which the array is constructed in an atmosphere having less than about 5 ppb ozone so that less than 10% of the nucleic acids are degraded over a period of 1 hour at a temperature of from 18°C to 25°C. Applicants have recognized that by carefully excluding ozone from the procedures involved in nucleic acid array preparation, as well as packaging, arrays can be prepared in which degradation of the nucleic acids is minimized, leading to improved and more sensitive arrays.

Again, the present invention is drawn to an improved method of preparing a nucleic acid array on a solid support. The synthesis steps are carried out in a non-oxidizing atmosphere. Applicants have provided comparative results that illustrate the decrease in signal obtained for oligonucleotides prepared by methods wherein oxidizing agents such as ozone are present. Indeed, the working Examples of the instant application demonstrate the dramatic effects of ozone exposure on probe yield. Applicants respectfully direct the Examiner to pages 22 to 24 of the specification and Figures 1 to 5 for details of these experiments, which are highlighted below.

Example1 (and Figure 1A) shows approximately 70% degradation of an 8-mer poly-T probe when exposed to ambient air having as little as 5 to 25 ppb ozone for 15 minutes.

Example 2 (and Figure 2) shows 50% degradation of an 16-mer at seven hours exposure to 20 ppb ozone.

Example 3 (and Figure 4) shows that exposure to pure nitrogen or oxygen still results in 15 to 20% degradation, exposure to unfiltered ambient air having 10 to 30 ppb ozone results in 50% degradation, and exposure to ultra pure air having an added 20 ppb ozone results in 85% degradation.

It is submitted that the art did not recognize the detrimental effects of even ppb levels of ozone on polynucleotide synthesis. The current inventors have both recognized the problem and provided a solution. This is the touchstone of patentability.

The Rejection under 35 U.S.C. §112

Claims 7 and 9 remain rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention.

The Examiner has expressed the view that it is not clear what the spatial orientation of the surface is relative to the light source. Yet, Applicants note that the Examiner has interpreted the claims as Applicants intended. In an effort to resolve this issue, Applicants have amended claims 7 and 9 to recite the language used by the Examiner in the Office Action of September 10, 2001. If the Examiner continues to have reservations regarding the clarity of claims 7 and 9, a telephonic interview may be appropriate.

Rejections under 35 U.S.C. §103(a)

The rejection of claims 4-6 under 35 U.S.C. §103(a) as being unpatentability over Terrett, Fodor and Urdea for reasons made of record in Paper No. 5 is maintained despite Applicants' amendment to explicitly recite that there is less than about 5 ppb ozone in the atmosphere. The rejection is respectfully traversed.

The rejection of claims 4-6 under 35 U.S.C. §103(a) as being unpatentable over Terrett, Fodor and Brennan for reasons made of record in Paper No. 5 is maintained despite Applicants' amendment to explicitly recite that there is less than about 5 ppb ozone in the atmosphere. The rejection is respectfully traversed.

The rejection of claims 1-3, 7-13 and 15 as unpatentable under 35 U.S.C. §103(a) over Terrett, Fodor and Urdea is newly presented. The rejection is respectfully traversed.

The rejection of claims 1-3, 7-13 and 15 as unpatentable under 35 U.S.C. § 103(a) over Terrett, Fodor and Brennan is newly presented. The rejection is respectfully traversed.

Because all four of these rejections have a common basis, Applicants will address them together.

The Examiner summarizes his position on page 7 of Paper No. 8 with the statement: "It would have been obvious .... to conduct automated nucleic acid synthesis as per Fodor et al. and Terrett in the presence of an inert gas." Applicants have no opinion on this conclusion since it does not address the invention as claimed. See MPEP §2143.03 for the proposition that all claim limitations must be taught or suggested by the prior art. The instant invention relies upon the exclusion of ozone; nowhere in the cited art is ozone even mentioned. No combination of the cited references can support an obviousness rejection.

Fodor, et al. was cited as disclosing a method for synthesizing oligonucleotides on a solid support. The Examiner then concluded that the use of certain reagents for the removal of protecting groups must mean the entire

synthesis is carried out in a non-oxidizing atmosphere, yet the Examiner can not point to any disclosure in Fodor suggesting the exclusion of ozone.

Terrett also is silent with respect to ozone concentrations in the solutions used during synthesis, storage or screening. Terrett fails to teach or *suggest* that ozone levels of less than about 5 ppb would provide improved arrays as produced by the presently claimed methods.

Urdea describes a multiple reactor system for polynucleotide synthesis in which reagents are moved throughout the system by the means of inert gases. The Examiner notes that it would have been obvious to conduct automated nucleic acid synthesis in the presence of an inert gas such as argon, nitrogen, helium or carbon-filtered air to protect the reagents from reacting with water. However, Table 1, columns 9 and 10 of Urdea describe the use of several reagents with aqueous solvent systems. In particular, oxidation steps are carried out with iodine in the presence of lutidine/THF/H<sub>2</sub>O (step 12) with steps 11 and 13 also utilizing water. A complete reading of Urdea fails to turn up a single mention of nucleic acid synthesis wherein an atmosphere having ozone levels of less than 5 ppb is used. In fact, the Urdea reference merely describes a system wherein reagents are moved around by the use of gases.

Brennan describes a method for preparing oligonucleotides wherein the reagents are moved via the use of an inert gas. The Examiner also notes that Brennan discloses at column 8, lines 40-55, that water and oxygen should be excluded from the reaction chamber in which phosphoramidites are used because phosphoramidites are sensitive to hydrolysis by traces of water and to oxidation by contact with air.

Applicants' invention goes significantly beyond the mere avoidance of water and ambient air during phosphoramidite coupling reactions. More particularly, claim 1 recites that "the synthesis steps are carried out in a non-oxidizing atmosphere" having less than 5 ppb ozone. The synthesis steps include deprotection steps, coupling steps, and further deprotection steps. In some embodiments, packaging of the oligonucleotide arrays is conducted in a facility that is controlled to minimize exposure to oxidants such as ozone (see claim 12). According to Brennan, only the phosphoramidite

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reagents are sensitive to water or air. As a result Brennan fails to provide that which is missing from the *prima facie* case allegedly set forth by the Examiner, namely motivation to carry out all synthesis steps in an atmosphere that is non-oxidizing, and in particular, has an ozone concentration of less than 5 ppb.

Still further, in each of Urdea and Brennan, the Examiner notes that inert gases are used to move reagents in a system to minimize exposure to air and water. However, neither of these references disclose or suggest that ozone should be excluded or minimized from the atmosphere. In fact, a word search of each of U.S. Patent Nos. 4,517,338 (Urdea), 5,424,186 (Fodor), and 5,814,700 (Brennan) conducted on the USPTO database, failed to turn up a single mention of ozone.

Nevertheless, the Examiner concludes that the existence of arrays that are fully functional in binding assays indicates that ozone concentrations did not adversely affect the libraries (see Paper No. 5, page 8, first full paragraph) and further concludes that the concentration of ozone in the production and storage of the arrays must have been in the range of about 0 to about 5 ppb. The Examiner has not met his burden of presenting objective evidence for his inference that the methods of Urdea and Brennan must have less than 5 ppb ozone. As stated in MPEP §2112, "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic." The Examiner must provide evidence tending to show inherency. Furthermore, Applicants have demonstrated that even pure nitrogen and oxygen environments produce some degradation of the array (See Figure 4).

In the absence of some recognition that decreasing ozone levels in the atmosphere would lead to the improved products provided by the presently claimed methods, Applicants submit that a *prima facie* case of obviousness has not been set forth by the Examiner and respectfully request that the rejections be withdrawn.

Applicants further note that the presentation of two new grounds of rejection requires the withdrawal of the finality of the Office Action in view of the fact the second new rejection could have been raised previously and was not necessitated by Applicants' amendment. See MPEP §706.07(a).

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The Examiner has not met his burden of providing objective evidence that the prior art teaches or suggests the preparation of arrays in an atmosphere having less than about 5 ppb ozone.

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (650) 752-2469

Respectfully submitted,



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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Claims**

Claims 7 and 9 have been amended as follows:

7. (Twice Amended) A method in accordance with claim 3, wherein said substrate is a planar support and is irradiated with light [directed from a source at a position opposite the surface comprising said] that passes through the surface from the side of the surface opposite the immobilized nucleotides.

9. (Twice amended). A method in accordance with claim 3, wherein said substrate is a planar support and is irradiated with light [directed from a source on the same side of the surface comprising said] that passes through the surface from the side of the surface opposite the immobilized nucleotides and said atmosphere is an inert gas atmosphere.